NIDA Optogenetics and Transgenic Technology Core (OTTC)
Protocol for genotyping LE-Tg(DAT-iCre)6Ottc transgenic rats
March 03, 2017

Prepare genomic DNA using the HotShot Method:

This method was adapted by NIDA-OTTC from the protocol originally described in *Biotechniques 29(1), 52-54 (2000). It has been used successfully with ear punches and* tail clips of rats.

Place the biopsy sample in 1.5ml microfuge tube.
Add 200 microliters of 50mM NaOH
Incubate tubes at 95C for 60 minutes.
Vortex tubes on medium power setting for 5 seconds.
Quick spin the tubes to bring down the condensation.
Neutralize each sample by adding 20 microliters of 1M Tris-HCl (pH 8).
Vortex tubes on medium power setting for 5 seconds.
Quick spin the tubes to bring down the condensation.

Debris (the "undigested" remnant of sample) may remain visible at the bottom of the tube. This is OK, but be sure to take only from the supernatant when setting the PCR reaction.

Prepare PCR reaction mix:

Master Mix Matrix	**1 reaction** (microliters)
2x One Taq mastermix	5.5
5 microM Forward Oligo 5 microM Reverse Oligo	1.1
Water	3.4
Total	10

Dispense 10ul per tube, then add 1ul of genomic DNA lysate.

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Specific Protocol for Genotyping LE-Tg(DAT-iCre)6Ottc rats:

Transgene-specific oligos for OTTC (iCre gene toDAT gene):

Oligo Name	Sequence (5' to 3')
iCRE F738	GTTCTGCCGGGTCAGAAAGAATGGT
DAT-R74923	GCACAGGTAGGGAAACCTCCAGACA

These oligos produce a 822 basepair amplicon, please see detail from EXP#YZ484

PCR program = 1taq_68
0. 94C – HOLD (hot start)
1. 94C – 2 min
2. 94C – 30 sec
3. 68C – 1 min
4. go to step 2, repeat 40x
5. 4C – HOLD (end of program)

Analyze PCR products

Run the samples on a 2% agarose gel in 1xTAE buffer.

Reagents List:

10M NaOH (Sigma)
1M Tris-HCl (pH 8) (Sigma-Aldrich)
OneTaq DNA polymerase mastermix (New England Biolabs)
Oligos (IDT DNA Technologies, standard synthesis and desalt preparation).

This protocol was updated on 03-03-2017 by Yajun Zhang.